5.0 DATA ON AR TA ASSAYS

5.1 Introduction

In vitro AR TA assay data and methodology information were collected from 26 publications and one submitted unpublished report on substances that had been evaluated *in vitro* for their ability to act as an AR agonist and/or antagonist. Where provided, the specific information extracted for each tested substance includes its name, source, purity, methodological details, and relevant data. If available, a CASRN was identified for each substance. This identifier was obtained from various sources, including the source publication, the National Library of Medicine's ChemID database, and *The Merck Index*. Chemical name synonyms were collected for substances that were identified in the literature by more than one name, and for substances where the name used in the publication may have been different from the generic name. All substances with the same CASRN were listed under the same name, usually the common name, regardless of the name that was used in the original publication. No attempt was made to identify the source and purity of a substance if the investigators did not provide such information. **Appendix C** provides information on the names, synonyms, CASRN, and chemical/product class, if identified, for each substance. **Appendix D** contains the *in vitro* AR TA assay data, organized by substance name, CASRN, and assay.

5.2 Availability of Detailed *In Vitro* AR TA Protocols

The Methods sections in the *in vitro* AR TA publications and the unpublished report provided various levels of detail. To the extent possible, the most relevant method parameters were extracted from each source and summarized in **Appendix A**. Details about the following method parameters are included in the Appendix to the extent this information was available:

- *Characteristics of the cell line* (e.g., name of cell line, its source).
- *Transfection of cells with plasmids* (e.g., identify whether transfections were stable or transient, AR expression vector, AR source, reporter vector, endpoint measured, plasmid transfected for cell toxicity measurements, endpoint measured for cell toxicity).
- *Preparation of cells for assay* (e.g., growth of cells before transient transfection, plating time prior to treatment of cells with a test substance).

- *TA assay* (e.g., identify whether assay evaluated agonism and/or antagonism, test substance solvent, test substance exposure duration, reference androgen, number of replicates per experiment, and number of times assay was repeated).

5.3 Availability of *In Vitro* AR TA Assay Data

In vitro AR TA assay data were collected on a total of 145 substances tested in the following AR reporter gene and cell proliferation assay systems:

- CHO cells stably transfected with an expression vector encoding hAR, and a reporter vector encoding luciferase linked to the mouse mammary virus tumor promoter (CHO hAR(S)+ Luc(S));
- CHO-K1 cells transiently transfected with an expression vector encoding hAR, and a reporter vector encoding luciferase linked to the mouse mammary virus tumor promoter and a third plasmid encoding (CHO-K1 hAR(T)+Luc(T)+EGFP(T));
- CHO-K1 cells stably transfected with an expression vector encoding hAR, and a reporter vector encoding luciferase linked to the mouse mammary virus tumor promoter (CHO-K1 hAR(S)+Luc(S));
- CHO cells transiently transfected with an expression vector encoding hAR, and a reporter vector encoding CAT linked to the mouse mammary virus tumor promoter and a third plasmid encoding β-gal (CHO hAR(T)+CAT(T)+β-gal(T));
- CHO cells transiently transfected with an expression vector encoding hAR, and a reporter vector encoding luciferase linked to the mouse mammary virus tumor promoter (CHO hAR(T)+Luc(T));
- CV-1 monkey kidney cells transiently transfected with vectors containing hAR cDNA and luciferase cDNA linked to MMTV (CV-1 hAR(T)+Luc(T));
- CV-1 monkey kidney cells transiently transfected with vectors containing hAR cDNA and CAT cDNA linked to MMTV (CV-1 hAR(T)+CAT(T));
- CV-1 monkey kidney cells transiently transduced with vectors containing hAR cDNA and luciferase cDNA linked to MMTV (CV-1 hAR(T)+Luc(T)*) (* refers to being transduced);
- HeLa human tumor cells stably transfected with the vector containing hAR cDNA and transiently transfected with luciferase cDNA (HeLa hAR(S)+Luc(T));

- HepG2 human hepatoma cells transiently transfected with vectors containing hAR cDNA and luciferase cDNA and β-gal (HepG2 hAR(T)+Luc(T)+β-gal(T));
- Human supraclavicular lymph node cells from prostate adenocarcinoma (LnCaP-FGC) containing an endogenous AR (LnCaP-FGC hAR(E)+CP);
- Human breast carcinoma cells containing endogenous AR and stably transfected with luciferase cDNA (MDA-MB-453-kb2 hAR(E)+Luc(S));
- Human breast carcinoma cells containing endogenous AR and transiently transduced with luciferase cDNA (MDA-MB-453 hAR(E)+Luc(T)*) (* refers to being transduced);
- Human prostate adenocarcinoma cells transiently transfected with vectors containing hAR cDNA and luciferase cDNA (PC-3 hAR(T)+Luc(T));
- Human prostate adenocarcinoma cells stably transfected with vectors containing hAR cDNA and luciferase cDNA (PALM hAR(S)+Luc(S));
- CV-1 monkey kidney cells transiently transfected with vectors encoding mouse AR and CAT (CV-1 mAR(T)+CAT(T));
- Carp skin tumor cells transiently transfected with vectors encoding rainbow trout ARα and CAT (EPC rtARα(T)+CAT(T)); and
- Yeast cells (*S. cerevisiae* or *S. cerevisiae YPH500*) stably transfected with hAR linked to a copper metallothionein promoter and a β-galactosidase expression vector (Yeast (*S. cer*) hAR(S)+β-gal(S))or (Yeast(*S. cer YPH500*) hAR(S)+β-gal(S)).

In studies that evaluated the potential agonism of a substance in an *in vitro* AR reporter gene assay, enzyme (i.e., luciferase; CAT; β-galactosidase) activity was used as an indirect measure of AR-induced transcriptional activation. To assess potency, enzyme levels induced by the test substance were typically compared to that produced by a reference androgen (DHT, R1881, testosterone, or mibolerone). The quantitative results of these *in vitro* AR TA studies were most commonly presented in terms of relative activity. However, the definition of relative activity varied greatly among the reports. Relative activity was expressed as:

- The fold induction of enzyme activity produced by the test substance relative to the activity in the untreated controls;
- The ratio of the response of the test substance to that of the reference androgen (sometimes termed relative potency);

- The concentration of the test substance that produced a certain percent response relative to the reference androgen; and
- The concentration of test substance that produced a specified fold-induction (e.g., 10-fold induction of enzyme activity) over background.

When provided, these various quantitative measures of agonism were extracted from the publications. Such data are provided in the "AGONISM Maximum Fold" column in **Appendix D**. Normalizing these values for comparison across the assays was not attempted. Instead, data from each study was assigned a qualitative response of positive or negative for the particular assay system (shown in the column named "AGONISM Qualitative" in **Appendix D**).

EC_{50} values were occasionally reported in the agonism studies. These values were extracted from the reports and are shown in the "AGONISM EC_{50}" column in **Appendix D**. The measures of EC_{50} were relative to the specific assay system used and were not compared across assays.

The antagonism studies using reporter gene expression systems measured the inhibition of reference androgen-induced enzyme activity by the test substance, and the IC_{50} value was often presented as a measure of response. These values are summarized in the "ANTAGONISM IC_{50}" column in **Appendix D**. In reports where an IC_{50} value was not provided but dose response data were presented, the IC_{50} values of the test substance and the reference androgen were estimated. These estimated IC_{50} values are italicized in **Appendix D**. For publications in which an IC_{50} value was not reported or a dose response curve not presented, test substances were assigned a qualitative response of positive or negative in the assay system used (shown in the "ANTAGONISM Qualitative" column in **Appendix D**).

Sonnenschein et al. (1989) used growth in a cell line that is dependent on androgens for replication as an *in vitro* measure of test substance-induced transcriptional activation. The investigators reported the study results in terms of RPP, which is the ratio (X 100) between the concentrations of the reference androgen (testosterone in this case) and the test substance necessary to induce maximal cell growth, as defined by the investigator. The RPP values for the substances tested were assigned a qualitative value of positive or negative (**Appendix D**).

5.4 *In Vitro* AR TA Assay Results for Individual Substances

The numbers of substances tested in each of the *in vitro* AR TA assays considered in this BRD are provided in **Table 5-1**. Of the 145 substances tested, only 23 (15.9%) were tested for agonism in three or more assays, irrespective of the reference androgen used. DHT was the most frequently tested substance in the AR TA agonism assays (15 assays), followed by 17β-estradiol and testosterone, which were tested in 12 and 11 assays respectively. Only 12 (8.3%) substances were tested in three or more antagonism assays, irrespective of the reference androgen used. The greatest number of different *in vitro* AR TA antagonism assays used to test the same substance was nine (for hydroxyflutamide).

A majority of the substances (91; 62.8%) were tested in only one laboratory for either agonism and/or antagonism. Among the *in vitro* AR TA assays included in this BRD, the assays that tested the greatest number of different substances, irrespective of testing for agonism or antagonism, were the CHO-K1 hAR(T)+Luc(T)+EGFP(T) assay (65 substances, 44.8%), the PALM hAR(S)+Luc(S) assay (43 substances, 29.7%), the Yeast (*S.cer*) AR+β-gal assay (32 substances, 22%), and the CV-1+hAR(T)+Luc(T) assay (27 substances, 18.6%).

5.5 Use of Coded Chemicals and Compliance with Good Laboratory Practice (GLP) Guidelines

Based on the available information, it appears that none of the *in vitro* AR TA studies used coded chemicals or complied with GLP guidelines.

Table 5-1 Substances Tested in Three or More *In Vitro* AR TA Assays Irrespective of the Reference Androgen Used

Substance	No. of Assays (agonism[a])	No. of Assays (antagonism)
DHT*	15	
17β-Estradiol	12	4
Testosterone*	11	
Progesterone	10	3
Hydroxyflutamide	6	9
Cyproterone acetate	6	5
Flutamide	6	5
R1881*	6	
Cortisol	5	
Dexamethasone	5	
Mifepristone	5	
p,p′-DDE	4	6
Diethylstilbestrol	4	
Medroxyprogesterone acetate	4	
2,2-Bis-(*p*-chlorophenyl)-1,1,1-trichloroethane (HPTE)	3	4
Aldosterone	3	
Estrone	3	
11-Ketotestosterone	3	
Levonorgestrel	3	
Methyltestosterone	3	
Mibolerone*	3	
p-Nonylphenol	3	
Norethisterone	3	
Bicalutamide		4
3',5'-Dichloro-2-hydroxy-2-methylbut-3-enanilide		4
2-[[3,5-(Dichlorophenyl)carbamoyl]oxy]-2-methyl-butenoic acid		4
Spironolactone		3
Vinclozolin		3

Abbreviations: *p,p′*-DDE = 1,1 Dichloro-bis[4-chlorophenyl]ethylene; DHT = 5α-Dihydrotestosterone; R1881 = Methyltrienolone.

[a] Includes the cell proliferation assay performed by Sonnenschein et al. (1989).

*Includes assays in which these substances were used as the reference ligand.